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IN VITRO CULTURE OF POINTED GOURD *(Trichosanthes dioica* Roxb.)

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Abstract

An experiment was conducted to study the *in vitro* culture of pointed gourd. Cotyledon rescued from physiologically matured seeds (PMS) and immatured seeds (IMS) of pointed gourd were used as explants. Cotyledon excised from PMS responded very well in all culture conditions. Plant regenerated from cotyledon of PMS ranged from 38 to 96% in different hormonal formulations of culture media. Highest percentage of shoot regeneration was observed in MS + 1.0 mg/l BAP and lowest in MS + 2.5 mg/l BAP. No plant regeneration was observed in cotyledon from immatured seeds. The highest percentage of root induction (99%) was achieved in half MS medium supplemented with 0.5 mg/l NAA. The regenerated plantlets were successfully established in earthen pot.

Keywords: Cotyledon, *in vitro*, pointed gourd.

Introduction

Pointed gourd *(Trichosanthes dioica* Roxb.) is an under exploited important summer vegetables in Bangladesh (Rashid, 1993). It is one of the most nutritive cucurbit vegetables that holds a coveted position in the vegetable market during summer and rainy season (Singh *et al.,* 1992). Being very rich in protein and Vitamin A, it has certain medicinal properties and many reports are available regarding its role in circulatory system, especially in lowering blood sugar and serum triglycerides (Sheshadri, 1990). In recent years, *in vitro* procedures are used to some degree in almost every major agronomic, vegetables, and fiber crops (Hoque *et al.,* 1998). The success of this technology requires an efficient protocol for plant regeneration from isolated organs, tissues, and cells (Islam *et al.,* 1994). The success of a crop improvement programme, depends on selection of desirable plants, which is possible if wide variation is present in the base population. But there is less variability in pointed gourd (Uddin, 2000), Variability can be created by somaclonal variation or by *in vitro* polyploidization (Hoque *et al.,* 1998). So, it is necessary to develop *in vitro* plant regeneration protocol for pointed gourd. But less attention has been given to tissue culture of pointed gourd than its closely related taxa, such as cucumber and melon (Dong and Jia, 1991). Plantlet formation has already been reported in *Cucurbita pepo*

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(Jelaska, 1974), water melon (Dong and Jia, 1991), cucumber (Chee, 1990; Gambley and Dodd, 1990) and *Momordica charantia* (Islam *et al.*, 1994). Therefore, the present investigation was conducted with a view to developing a protocol for plant regeneration through *in vitro* culture of pointed gourd.

Materials and Method

The study was conducted in the Tissue Culture Laboratory, Biotechnology Division, BARI, Joydebpur, Gazipur 1701. The experiments were conducted following Completely Randomized Design (CRD). The data were subjected to mean value plus Standard Error (S. E).

Plant materials

Cotyledon rescued from matured (PMS) and immatured seeds (IMS) of pointed gourd were considered as explants for this study. The explants were collected from the experimental field of pointed gourd, Plant Genetic Resources Centre (PGRC), BARI, Joydebpur, Gazipur 1701.

Preparation of explant

Fourteen to seventeen days old fruits of pointed gourd having physiologically matured seeds (PMS) with solid cotyledons were collected from the experimental field of pointed gourd, PGRC, BAR!, Gazipur. The fruits were cut and the seeds removed from the fruits with the help of sterilized scalpel. The seed coats were removed carefully with the help of sterilized forceps and knife and the cotyledons were taken in a beaker. Eight to twelve days old fruits having IMS with soft seed coat and semi solid cotyledons were collected from the same experimental field and prepared according to the previous methods.

Surface sterilization of explant

Prepared cotyledons were surface sterilized gently with distilled water for 3-4 times. The surface sterilized cotyledons were dipped into 70% ethyl alcohol for 30 seconds. Finally, the cotyledons were washed 3 times with sterilized distilled water inside the clean bench and these cotyledons (excluding embryo) were placed in the culture medium.

Inoculation

The excised solid and semi-solid cotyledons (excluding embryo) were inoculated in each culture test tubes containing MS media with various concentrations of BAP (0.5, 1.0, 1.5. 2.0, and 2.5 mg/l) and NAA (0.5, 1.0, 1.5. 2.0, and 2.5 mg/l) for *in vitro* shoot regeneration. The pH of the medium was adjusted to 5.7 ± 0.1 using 0.1 N sodium hydroxide (NaOH) or 0.1 N HCI. In order to solidify the media, laboratory grade agar of 8.0 g (0.8%) was added to the solution. The

culture tubes were plugged with aluminium foil and marked with glass marker pen to indicate specific hormonal supplement. The culture tubes were sterilized at 1.09 kg/cm^2 pressure at 121°C for 15 minutes in an autoclave. After auto claving, the culture media were taken out and allowed to cool and solidify. For growth and development of cultures, the temperature was set 25±1°C at a light intensity of 2000-3000 lux from fluorescent tubular lamps and the photoperiod was maintained generally 16 hours light and 8 hours dark (16 L/8 D) and relative humidity 60-70%.

Successful shoot formation become evident when small green fresh leaves began to emerge. Subcultures carried out regularly at an interval of 4-5 weeks. The shoot induction percentages, days to shoot initiation, shoot number per explant, and shoot length have been recorded after 4 weeks of culture. *In vitro* grown shoots of pointed gourd were cultured in $^1/_2$ MS medium supplemented with different concentration of NAA (0.1, 0.2, 0.3, 0.4, and 0.5 mg/l) for root initiation. The well rooted plantlets were then kept in room temperature for 2-3 days and transferred to polyethylene bags containing a mixture of soil, sand, and rice bran ash (1:1:1) and moist them adequately for proper hardening.

Results and Discussion

The choice of explants is of cardinal importance and makes an absolute difference between success and failure in inducing regeneration *in vitro.* In most of the cucurbits, the root induction was achieved on either basal MS medium alone or with very low level of auxin (Mythili and Thomas, 1999). In the present experiment, different concentrations of BAP and NAA were used singly to investigate the induction of shoot and its subsequent regeneration. Data on shoot induction percentage, days to shoot initiation, shoot number per explant and shoot length per explant were recorded after 4 weeks of culture. The results are presented under the following separate heads:

Shoot induction and proliferation

The response of cotyledon explants from matured and immatured seeds of pointed gourd cultured in MS media supplemented with BAP (0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) and NAA (0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) are shown in Table 1. The cotyledon obtained from matured seeds when used as the explant produced roots and shoots. The highest percentage of shoot induction (96.00%) and highest number of shoot per explant (2.55) were obtained in MS medium supplemented with 1.0 mg/l BAP followed by 1.0 mg/l NAA (82.00%, 2.30) and the lowest percentage of shoot induction (40.00%) recorded in 2.5 mg/l BAP. The higher shoot formation was observed in MS + 1.0 mg/l BAP. The results proved that BAP has a positive effect on shoot induction. Shoot development occurred within 14 days in 1.0 mg/l BAP, whereas it took 17 days in 1.0 mg/l NAA. Similar

results were reported by Hoque *et al.* (1998) in pointed gourd. They also observed roots and shoots in hormone free MS medium which is not similar to the results observed in this experiment.

Table 1. Effect of different concentrations of BAP and NAA on shoot induction and proliferation from cotyledon of matured and immatured seeds of pointed gourd.

Explant	Treatment (mg/l)		Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)
Cotyledon from matured seeds	Control		0	0	0	0
	BAP	0.5	65.00	14	2.20*	3.72 **
		1.0	96.00 **	14	2.55 **	3.81**
		1.5	55.00	14	2.00	3.10
		2.0	41.00	14	1.50	2.00
		2.5	40.00	14	1.00	1.45
	Mean		59.40	14.00	1.85	2.82
	S.E(±)		9.176	0.000	0243	0.420
Cotyledon from matured seeds	Control		0	0	0	0
	NAA	0.5	68.00	17	2.10	3.31 **
		1.0	82.00 **	17	2.30 **	3.57 **
		1.5	56.00	17	2.27	2.56
		2.0	47.00	17	2.00	2.20
		2.5	43.00	17	1.48	2.10
	Mean		59.20	17.00	2.03	2.74
	S.E(±)		6.383	0.000	0.132	0.264
Cotyledon from immatured seeds	Control		0	0	0	0
	BAP	0.5	0	0	0	0
		1.0	0	0	0	0
		1.5	0	0	0	0
		2.0	0	0	0	0
		2.5	0	0	0	0
Cotyledon from immatured seeds	Control		0	0	0	0
	NAA	0.5	0	0	0	0
		1.0	0	0	0	0
		1.5	0	0	0	0
		2.0	0	0	0	0
		2.5	0	0	0	0

*, ** Significant at 5% and 1% level, respectively.

Semi-solid cotyledon obtained from immatured seeds did not produce any root and shoot. The cell mass of immatured cotyledon may not be treated as a suitable explant for regeneration.

Shoot multiplication

Multiplication of regenerated shoot is the prime objective of the *in vitro* plant regeneration or multiplication of plant species. The hormonal concentrations had a significant effect on multiple shoot regeneration. In this experiment, different concentrations of BAP were used along with control in 1/2 strength MS basal medium to study their effect on shoot induction and multiple shoot formation from nodal segments of *in vitro* grown plantlets of pointed gourd. Results are presented in Table 2. The best result was recorded in 1/2 MS + 2.0 mg/l BAP when nodal segment was used as explants of *in vitro* grown plantlets (Table 2.). Significantly highest percentage of the explants developed shoot (98.00%), highest multiple shoots per explant (5.50*)* and the longest shoot per explant (6.55 cm) were observed in 2.0 mg/l BAP when the nodal segment of *in vitro* grown plantlets cultured in MS medium followed by 1.5 mg/l BAP (95.00%, 4.00 and 5.05 cm, respectively). The lowest percentage of shoot induction (80.00%) was

Table 2. Effect of different concentrations of BAP in half strength MS medium on shoot multiplication from nodal segment and shoot tip of *in vitro* plantlets of pointed gourd.

Explant	Treatment (mg/l)	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length per explant (cm)
	Control	0	0	0	0
	BAP				
	0.5	80.00	11	3.50	4.10
	1.0	85.00	11	3.67	4.17
	1.5	95.00*	11	4.00	5.05*
	2.0	98.00**	11	5.50**	6.55**
	2.5	90.00	11	3.52	3.50
	Mean	89.60	11.00	4.03	4.67
	S.E(±)	2.920	0.000	0.336	0.474
Shoot tip	Control	0	0	0	0
	BAP				
	0.5	70.00	11	3.00	3.40
	1.0	74.00	11	3.20	3.45
	1.5	81.00*	11	3.51	4.35*
	2.0	88.00**	11	4.25**	4.85**
	2.5	78.00	11	3.15	3.50
	Mean	78.20	11.00	3.42	3.87
	S.E(±)	2.74	0.000	0.199	0.277

*, ** Significant at 5% and 1% level, respectively.

found in MS + 0,5 rng/l BAP. When shoot tip explants of *in vitro* grown plantlets of pointed gourd cultured in 1/2 MS media supplemented with 2.0 mg/l BAP, the highest shoot induction percentage (88.00%), maximum number of shoots (4.25)

and the longest shoot (4.85 cm) were also observed (Table 2) followed by $^{1}/_{2}$ MS + 1.5 mg/l BAP (81.00%, 3.51, and 4.35 cm for shoot induction percentage, shoot number per explant and shoot length per explant, respectively). The lowest percentage of shoot induction (70.00%) was found in MS + 0.5 mg/l BAP. The nodal segments showed shoot induction percentage (98.00%), shoot number per explant (5.50) and shoot length per explant (6.55 cm) which were less compared to shoot tips explants for all the characters studied. The results indicated that nodal explants were more capable of producing multiple shoots compared to those of the shoot tip explants. These results were in agreement with the findings of Debnath *et al.* (2000) and Uddin (2000) in pointed gourd. Zaman *et al.* (1992) demonstrated similar effects of BAP on shoot elongation in nodal segments culture of *Verbena* spp. (Hosoki and Katahira, 1994). The greater responsiveness of nodal explants over shoot apices can be attributed to the absence of apical dominance and the presence of axillary buds at a more advanced stage of development. It may be mentioned here that the shoot apex displays apical dominance, which might result from auxin produced at the terminal bud. Due to apical dominance, the lateral bud formation is suppressed. In apple (Hutchinson, 1981) and thornless blackberry (Zimmerman and Broome, 1980) nodal segments proved to be good explants for micro propagation. It was observed that MS medium without hormone had no response in shoot regeneration for all the explants of pointed gourd.

Table 3. Effect of NAA in half strength MS medium on rooting of induced shoots in pointed gourd.

Treatment (mg/l)		Days to root initiation	Root induction (%)	Root number per explant	Root length (cm)
Control		0	0	0	0
NAA	0.1	17	55	7	6.20
	0.2	17	60	9	6.30
	0.3	17	72	9	7.15
	0.4	14	80	10	7.45**
	0.5	14	99**	12**	7.20
Mean		15.80	73.20	9.40	6.86
S.E(±)		0.735	7.806	0.812	0.569

*, ** Significant at 5% and 1% level, respectively.

Rooting of *in vitro* grown shoot

Easy and high frequency rooting is very important for establishment of *in vitro* regenerated plantlets. For successful micro propagation, healthy and strong root system is required. Response of different concentrations of NAA in half strength MS medium for *in vitro* adventitious root formation is presented in Table 3. The

highest percentage of root formation (99.00%) and number of roots per explant (12) were found in 0.5 mg/l NAA which was significantly different from other treatments. But longest root per explant (7.45 cm) was observed in 0.4 mg/l NAA. Early root induction was obtained from the treatment of 0.4 mg/l NAA and 0.5 mg/l NAA. This result was in agreement with the findings of Mamun Hossain *et al.*, 1996 and Uddin, 2000.

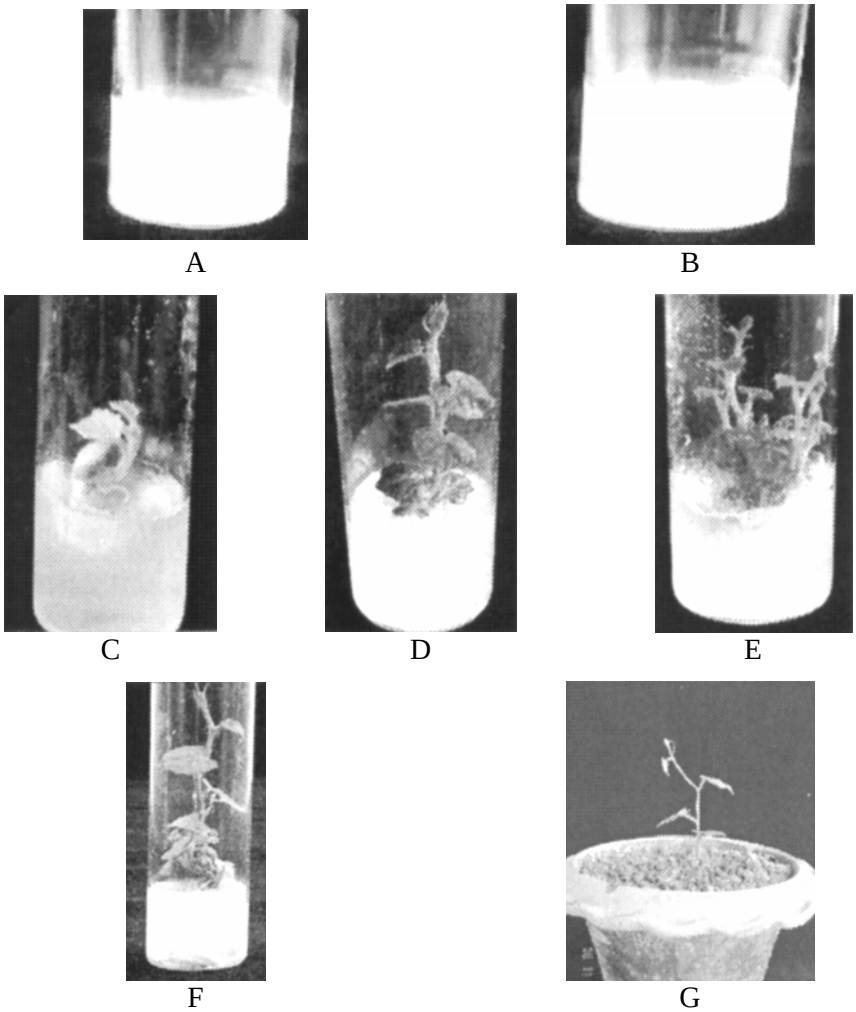

Fig. 1. Plant regeneration of pointed gourd through cotyledon
A & B. No. Shoot initiation from IMS
C. Shoot initiation from PMS
D. Shoot formation from PMS
E. Multiple shoots from PMS
F. Rooted shoot from PMS
G. Established plant in earthen pot

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